RHÔNE-POULENC Contains No CBI

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RHÔNE-POULENC INC.
CN 7500, CRANBURY, NJ 08512-7500
TELEPHONE: (609) 395-8300

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Document Processing Center (TS-790)
Attn: Section 8(e) Coordinator (CAP Agreement)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

RE: Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

CAP ID NO.: 8ECAP - 0004

RP CAP REPORT NO.: RPS - 0176

Dear Sir/Madam:

On behalf of Rhône-Poulenc Inc. (RPI, CN5266, Princeton, NJ 08543-5266) and its subsidiaries, the attached report is being submitted to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program (CAP Agreement) executed by RPI and EPA (8ECAP - 0004).

The enclosed report provides information on the following chemical substance:

Chemical Identity:	Glyceryl propoxy triacrylate (GPTA) (Coded as C-486 in report)
CAS Registry No:	52408-84-1
CAS Registry Name:	Poly[oxy(methyl-1,2-ethanediyl)], α,α',α"-1,2,3-propanetriyltris[ω-[(1-oxo-2-propenyl)oxy]-

NVM
3/10/95

The title of the enclosed report is:

> Mutagenicity Evaluation of C-486 in the Mouse Lymphoma Forward Mutation Assay

The following is a summary of the adverse effects observed in this report.

> This study is being reported under Section 8(e) CAP because acrylates generally produce positive results in the mouse lymphoma assay. In this study, significant increases in mutant frequency were observed at concentrations of 1.0 nl/ml and higher without activation and 15.0 nl/ml and higher with activation.

RPI does not claim any portion of the information in this submission to be TSCA confidential business information (TSCA CBI).

RPI has not previously submitted any TSCA Section 8(e) notices or premanufacture notification on the subject chemical substance.

RPI has submitted other studies on this material under the CAP Agreement; see RP CAP Report Nos. RPS-0177 and RPS-0273.

On August 15, 1985, Celanese submitted to EPA all available toxicity data on the multifunctional acrylates. However, RPI does not have a detailed list in our records of the reports that were submitted. Therefore, RPI is submitting three copies of the enclosed report and this cover letter: an original and two copies.

Further questions regarding this submission may be directed to Dr. Glenn S. Simon, Director of Toxicology at (919)549-2222 (Rhône-Poulenc, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709).

Sincerely,

Charles E. Moyer, Jr., Ph.D.
Director, Product Safety
(609)860-3589

CEMjr/mm
Enclosures

GENETICS ASSAY NO.: 6837

LBI SAFETY NO.: 8396

3

CAP ID No. S-LT-PGN-0067
Reviewed for Sec. 8 (e)
Compliance Program
On 10/7/91 By

MUTAGENICITY EVALUATION OF

C-486

IN THE
MOUSE LYMPHOMA FORWARD
MUTATION ASSAY

FINAL REPORT

SUBMITTED TO:

CELANESE CORPORATION
1211 AVENUE OF THE AMERICAS
NEW YORK, NEW YORK 10036

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO.: 20989

REPORT DATE: MAY, 1983

4

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains Items I-IX. Items I-IV provide sponsor and test article identification information, type of assay, and the protocol reference number. Item V provides the initiation and completion dates of the study. Item VI identifies the supervisory personnel. Item VII indicates the tables and/or figures containing the test results. The interpretation of the results is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the study design, which includes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices.

All test and control results presented in this report are supported by raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895.

Copies of the raw data will be supplied to the sponsor upon request.

The described study was performed in accordance with Good Laboratory Practice regulations except if noted to the contrary. To the best of the signer's knowledge there were no significant deviations from the Good Laboratory Practice regulations which affected the quality or integrity of the study.

I. SPONSOR: Celanese Corporation

II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NO.: 6837

A. Identification: C-486

B. Date Received: March 10, 1983

C. Physical Description: Clear, pale yellow liquid

III. TYPE OF ASSAY: Mouse Lymphoma Forward Mutation Assay

IV. ASSAY DESIGN NUMBER: 431, Edition 5

V. STUDY DATES

A. Initiation Date: March 18, 1983

B. Completion Date: May 2, 1983

VI. SUPERVISORY PERSONNEL:

A. Study Director: Maria A. Cifone, Ph.D.

B. Laboratory Supervisor: Violeta V. Balinas

VII. RESULTS:

The data are presented in Table 1 on page 4.

VIII. INTERPRETATION OF RESULTS:

The test material, C-486, formed a clear, colorless liquid in dimethylsulfoxide (DMSO) at 100 µl/ml. Just prior to each assay, stock solutions were prepared by performing serial dilutions in DMSO. The cytotoxicity and mutation assays were then initiated by performing final 1:100 dilutions of the stocks into media containing the cells. The test material appeared soluble in the assay media up to 500 nl/ml but a small amount of precipitate was apparent at 1000 nl/ml. In the preliminary cytotoxicity assay, the test material was lethal at 31.3 nl/ml without activation and lethal at 125 nl/ml with activation.

One trial of the mutation assay was performed and the results are shown in Table 1.

Under nonactivation conditions, the test material was assayed for mutant induction from 0.5 nl/ml to 3.0 nl/ml and moderate to high toxicities were induced (percent relative growths, 40.7% to 4.9%). A dose-dependent increase in the mutant frequency was induced. The minimum

VIII. INTERPRETATION OF RESULTS: (continued)

criterion for mutagenesis in this assay was a mutant frequency exceeding 55.6×10^{-6}. Treatments from 1.0 nl/ml to 3.0 nl/ml induced mutant frequencies that exceeded the minimum criterion and the active treatments induced mutant frequencies ranging from 56.5×10^{-6} to 232.4×10^{-6}. The test material was therefore considered mutagenic without activation in this assay.

In the presence of metabolic activation mix, the test material was converted to a less toxic form or forms and treatments from 2.5 nl/ml to 60 nl/ml were assayed for mutant induction. In order for a treatment to be considered mutagenic in this assay, a mutant frequency exceeding 82.0×10^{-6} was required. Five of the six assayed treatments induced mutant frequencies that exceeded the minimum criterion. The increases ranged from 3.6-fold to 8.2-fold above the background mutant frequency (average of solvent and untreated controls). The test material was therefore considered mutagenic with activation in this assay.

In the assays used in this evaluation, the average cloning efficiencies for the solvent and untreated negative controls varied from 58.6% without activation to 74.2% with activation which demonstrated acceptable cloning conditions for the assays. The negative control mutant frequencies were all in the normal range and the positive control compounds yielded normal mutant frequencies that were greatly in excess of the background.

IX. CONCLUSIONS:

The test material, C-486, induced significant increases in the mutant frequency at the TK locus in L5178Y mouse lymphoma cells. Treatments up to 3.0 nl/ml without activation and up to 60 nl/ml with activation were assayed and a wide range of toxicities was induced. Dose-dependent increases in the mutant frequency were induced with and without activation. The increases ranged from 1.9-fold to 8.2-fold above the background mutation frequency. The active treatments occurred at moderate and high toxicities. The test material is therefore considered active in the Mouse Lymphoma Forward Mutation Assay with and without activation.

SUBMITTED BY:

Study Director

Maria A. Cifone

Maria A. Cifone, Ph.D.
Cell Biologist
Department of Molecular Toxicology

5-7-83
Date

REVIEWED BY:

Brian C. Myhr

Brian C. Myhr, Ph.D.
Director
Department of Molecular Toxicology

5/27/83
Date

SUMMARY OF MOUSE LYMPHOMA (L5178Y) RESULTS — TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: C-486
B. GENETICS ASSAY NO: 6837
C. SOLVENT: DMSO
D. SELECTIVE AGENT: TFT 3 UG/ML
E. TEST DATE: 04/18/83

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY		RELATIVE GROWTH (%)	MUTANT FREQUENCY (10E-6 UNITS)
NONACTIVATION				AVG SOLV CONTROL				AVG SOLV CONTROL		
SOLVENT CONTROL	12.1	11.0	14.8		46.0	184.0	61.3		100.0	25.0
SOLVENT CONTROL	13.0	12.9	18.6	16.7	65.0	180.0	60.8	60.7	100.0	36.1
UNTREATED CONTROL	11.9	8.7	11.5		49.0	163.0	54.3		61.7	30.1
EMS 0.25 UL/ML	8.2	11.2	10.2		522.0	108.0	33.3		33.5	522.0
EMS 0.4 UL/ML	9.3	7.1	7.3		530.0	74.0	24.7		17.8	716.2
TEST COMPOUND			RELATIVE TO SOLV CONTROL (%)				RELATIVE TO SOLV CONTROL (%)			
0.5000 NL/ML	8.0	6.1	32.5		70.0	206.0	113.1		36.8	34.0
0.7500 NL/ML	8.7	6.6	38.2		49.0	194.0	106.5		40.7	25.3
1.0000 NL/ML	5.8	6.8	26.2		83.0	147.0	80.7		21.1	56.5
1.5000 NL/ML	6.1	6.7	27.2		158.0	117.0	64.3		17.5	135.0
2.0000 NL/ML	5.1	5.6	19.0		216.0	152.0	83.5		15.9	142.1
3.0000 NL/ML	4.0	3.3*	8.8		258.0	111.0	55.4		4.9	232.4
S9 ACTIVATION	BATCH NO: RFH130			AVG SOLV CONTROL				AVG SOLV CONTROL		
SOLVENT CONTROL	8.6	16.9	16.1		122.0	199.0	66.3		100.0	61.3
SOLVENT CONTROL	9.6	14.8	15.8	16.0	86.0**	244.0	81.3	73.8	100.0	35.2
UNTREATED CONTROL	9.4	15.1	15.8		107.0	225.0	75.0		100.2	47.6
DMN 0.15 UL/ML	8.3	9.5	8.8		255.0	120.0	40.0		29.7	212.5
DMN 0.3 UL/ML	7.9	8.7	7.6		248.0	75.0	25.0		16.2	330.7
TEST COMPOUND			RELATIVE TO SOLV CONTROL (%)				RELATIVE TO SOLV CONTROL (%)			
2.5000 NL/ML	9.5	14.7	97.0		132.0	202.0	91.2		88.5	65.3
15.0000 NL/ML	7.9	13.9	76.3		243.0	142.0	64.1		48.9	171.1
20.0000 NL/ML	6.0	14.0	58.3		289.0	165.0	74.5		43.4	175.2
30.0000 NL/ML	6.8	7.9	37.3		402.0	177.0	79.9		29.8	227.1
50.0000 NL/ML	3.0*	4.9	10.2		367.0	94.0	42.5		4.3	390.4
60.0000 NL/ML	1.5*	3.8*	7.9		479.0	121.0	43.1		3.4	395.9

CULTURES ARE NORMALLY SPLIT BACK TO 3 X 10E5 CELLS/ML AND 3 X 10E6 CELLS ARE SEEDED FOR MUTANT SELECTION AND 300 CELLS SEEDED FOR VIABLE COLONY COUNT. IF THE DAILY COUNT IS MARKED BY (*), THE NUMBER OF CELLS SEEDED FOR MUTANT SELECTION WAS THE PRINTED COUNT VALUE TIMES 10E6 AND THE NUMBER SEEDED FOR VIABLE COLONIES WAS THE PRINTED COUNT VALUE TIMES 100

SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK) * (DAY 3 COUNT)/(3 OR DAY2 COUNT IF NOT SPLIT BACK)

MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 13E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 13E-6

CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED

RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

* NOT SPLIT BACK

**ONE PLATE CONTAMINATED; TOTAL CALCULATED USING ASSUMED VALUE FOR THE LOST PLATE, BASED ON AVERAGE OF TWO REMAINING PLATES

ASSAY DESIGN NO. 431

CHANGE SHEET

The positive control, ethylmethanesulfonate (EMS), is usually assayed at a concentration of 0.5 µl/ml (Part 3C.2). The EMS has been more active in recent studies, therefore the concentration was reduced to as low as 0.25 µl/ml to maintain the mutant frequencies in the range of the historical data.

MOUSE LYMPHOMA FORWARD MUTATION ASSAY
ASSAY DESIGN NO. 431, EDITION 5

1. OBJECTIVE

The mouse lymphoma forward mutation assay evaluates a test article's mutagenic potential in a specific locus mutation assay using mammalian cells. The objective of the assay is to determine the ability of a test article to induce forward mutations at the thymidine kinase (TK) locus as assayed by colony growth of L5178Y TK+/- mouse lymphoma cells in the presence of 5-bromo-2'-deoxyuridine (BrdU) or 5-trifluorothymidine (TFT).

2. RATIONALE

Thymidine kinase (TK) is a cellular enzyme that allows cells to salvage thymidine from the surrounding medium for use in DNA synthesis. If a thymidine analog such as BrdU is included in the growth medium, the analog will be phosphorylated via the TK pathway and be incorporated into DNA, eventually resulting in cellular death. Cells which are heterozygous at the TK locus (TK+/-) may undergo a single step forward mutation to the TK -/- genotype in which little or no TK activity remains. Such mutants are as viable as the heterozygotes in normal medium because DNA synthesis proceeds by de novo synthetic pathways that do not involve thymidine as an intermediate. The basis for selection of the TK-/- mutants is the lack of any ability to utilize toxic analogs of thymidine, which enables only the TK-/- mutants to grow in the presence of BrdU. Cells which grow to form colonies in the presence of BrdU are therefore assumed to have mutated, either spontaneously or by the action of a test substance, to the TK-/- genotype.

3. MATERIALS

A. Indicator Cells

The mouse lymphoma cell line, L5178Y $TK^{+}/_{-}$ - 3.7.2C, used in this assay was derived from the Fischer L5178Y line of Dr. Donald Clive. Stocks are maintained in liquid nitrogen and laboratory cultures are periodically checked for the absence of mycoplasma contamination by culturing methods. To reduce the negative control frequency (spontaneous frequency) of TK-/- mutants to as low a level as possible, cell cultures are exposed to conditions which select against the TK-/- phenotype (exposure to methotrexate) and are then returned to normal growth medium for three or more days before use.

B. Media

The cells are maintained in Fischer's mouse leukemia medium supplemented with pluronic solution, L-glutamine, sodium pyruvate, antibiotics, and horse serum (10% by volume). Cloning medium consists of the preceding growth medium minus pluronic, with the addition of agar to a final concentration of 0.35% to achieve a semisolid state. Selection medium is cloning medium containing 100 µg/ml of BrdU or 3 µg/ml of TFT.

3. MATERIALS (continued)

C. Control Compounds

1) Negative Controls

A negative control consisting of assay procedures performed on untreated cells is performed in all cases. If the test compound is not soluble in water, an organic solvent (normally DMSO) is used; the final concentration of an organic solvent in the growth medium will be 1% or less. Cells exposed to solvent in the medium are also assayed as the solvent negative control to determine any effects on survival or mutation caused by the solvent alone. For test substances assayed with activation, the untreated and solvent negative controls include the activation mixture.

2) Positive Controls

Ethylmethane sulfonate (EMS) is highly mutagenic via alkylation of cellular DNA and is used at 0.5 µl/ml as a positive control for non-activation studies.

Dimethylnitrosamine (DMN) requires metabolic activation by microsomal enzymes to become mutagenic and is used at 0.1 to 0.3 µl/ml as a positive control for assays performed with activation.

D. Sample Forms

Solid materials are dissolved in water, if possible, or in DMSO, ethanol or acetone unless another solvent is requested. Liquids are tested by direct addition to the test system at predetermined concentrations or following dilution in a suitable solvent.

4. EXPERIMENTAL DESIGN

A. Dose Selection

The solubility of the test article in water and/or an organic solvent is determined first. The range of test article concentrations assayed for mutant induction is determined during the mutation assay. At the discretion of the study director, a preliminary cytotoxicity test is performed. A wide range of test article concentrations is tested for cytotoxicity, starting with a maximum applied dose of 10 mg/ml (or 10 µl/ml) for water soluble articles or 1 mg/ml (or 1 µl/ml) for articles in organic solvents and followed by two-fold dilution steps. After an exposure time of 4 hours, the cells are washed and a viable cell count is obtained the next day. Relative toxicities, expressed as the reduction in growth of untreated cells, are used to select 7 to 10 doses for mutagenicity testing which represent a reduction in 24-hour growth that ranges from 0 to 50-90%.

4. A. Dose Selection (continued)

These selected doses are prepared for mutagenicity testing, but only 4 or 5 of the doses are carried through the mutant selection process. This procedure compensates for daily variations in cellular cytotoxicity. The cultures and cloning dishes are labeled according to SOP Number 522.

B. Mutagenicity Testing

1) Nonactivation Assay

The procedure used is based on that reported by Clive and Spector (1975) and is summarized as follows. Ten to 15 doses are exposed to the test chemical for four hours and are then washed and placed in growth medium for two or three days to allow recovery, growth and expression of the induced TK-/- phenotype. Cell counts are determined daily and appropriate dilutions are made to allow optimal growth rates. At the end of the expression period, 5 doses are usually selected for mutant analysis.

After the doses for cloning are chosen, 3×10^6 cells for each selected dose are seeded in soft agar plates with selection medium; resistant (mutant) colonies are counted after approximately 10 days incubation. To determine the actual number of cells capable of forming colonies, a portion of the cell suspension is also cloned in normal medium (nonselective). The ratio of resistant colonies to total viable cell number is the mutant frequency.

A detailed flow diagram for the mutation assay is provided in Figure 1.

2) Activation Assay

The activation assay can be run concurrently with the nonactivation assay. The only difference is the addition of the S9 fraction of rat liver homogenate and necessary cofactors (CORE) during the four-hour treatment period. CORE consists of NADP (sodium salt) and isocitric acid.

3) S9 Homogenate

A 9,000 x g supernatant prepared from adult male rat liver induced by Aroclor 1254 (described by Ames et al., 1975) is commercially prepared for use in this assay.

5. REPORT

The screened doses, cell counts, and mutant and viable colony counts will be entered into a computer program. The results are analyzed and printed.

The suspension growth of each culture is calculated as (Day 1 Cell Count/3) x (Day 2 Cell Count/3) x (Day 3 Cell Count/3) when the cultures are split back to 3 x 10^5 cells/ml after the daily count. If the cell count is less than 4 x 10^5 cells/ml, the culture is not split back and the cell count is substituted for 3 in the denominator of the next daily count. In most assays, 3-day expressions are not used, so only the first two factors in the preceding calculation are used. The suspension growth is calculated for each solvent control and then averaged. Relative suspension growth values are derived by dividing the suspension growth values by the average solvent control value and multiplying by 100%.

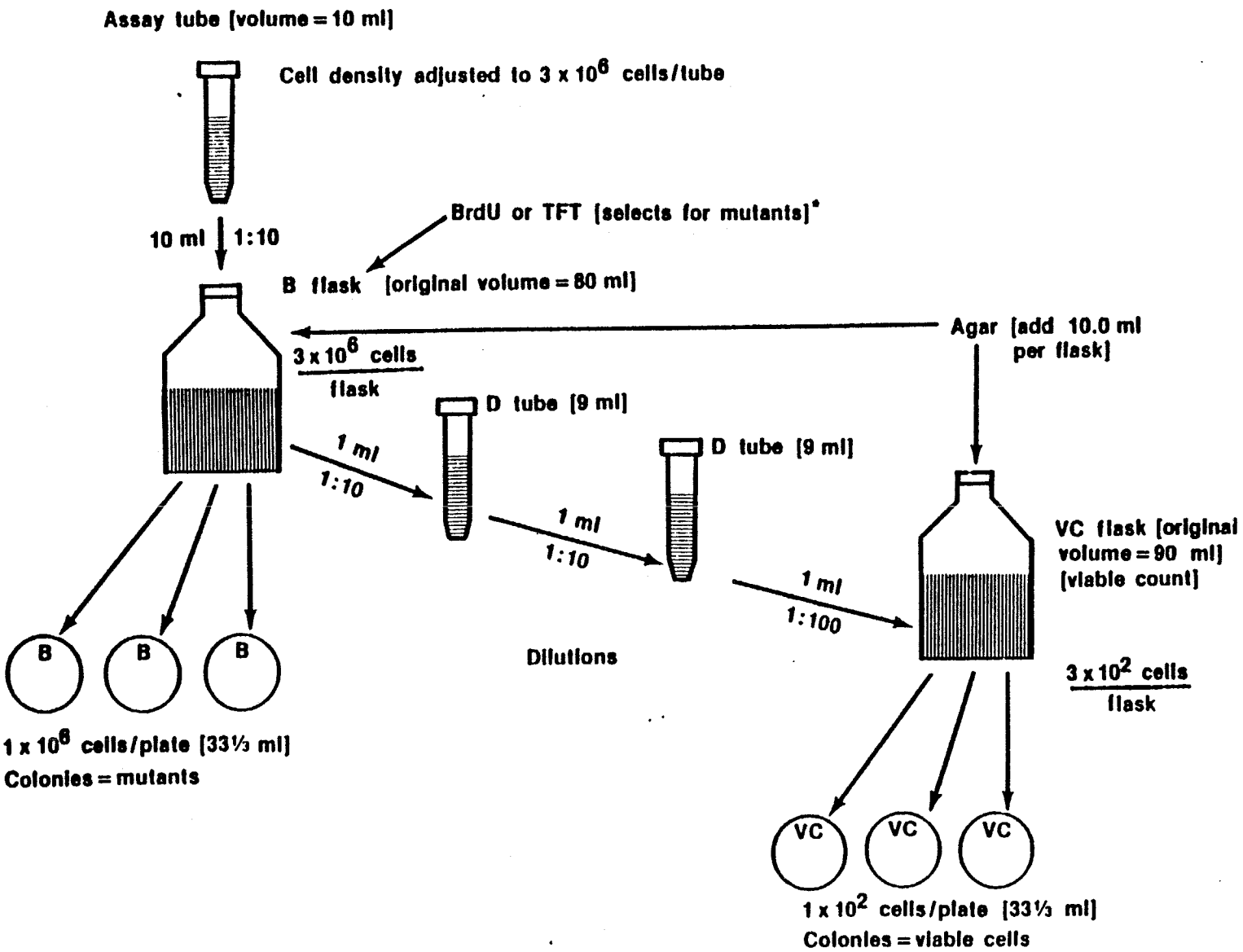
The average cloning efficiency for the negative controls in an assay is the average number of viable colonies for the solvent and untreated controls, divided by 300 and multiplied by 100%. In the computer tables, the cloning efficiency of each culture is expressed relative to the average solvent control cloning efficiency. Whenever the number of cells seeded for viable colony counts differs from 300, the computer calculation of the relative cloning efficiency is adjusted by the factor (300/cells seeded).

A percent relative growth value is calculated as (relative suspension growth) x (relative cloning efficiency/100). Corrected values for the relative cloning efficiency are used in the cases where the number of cells seeded for viable colonies differs from 300.

The mutant frequency is calculated as the ratio of mutant colonies to viable colonies times 10^{-4}. This calculation is unaffected by changes in the number of cells seeded for viable count because the number of cells seeded for mutant selection is changed by the same factor. Thus, as an example, if 250 cells are seeded for viable count, 2.5 x 10^6 cells are seeded for mutant selection; the 10^{-4} factor remains constant.

6. REFERENCE

Clive, D. and Spector, J.F.S.: Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Res., 31:17-29, 1975.



*Added after removal of 1 ml for viable count dilutions.

FIGURE 1 LYMPHOMA CLONING FLOW CHART

ASSAY ACCEPTANCE CRITERIA

An assay will normally be considered acceptable for evaluation of the test results only if all of the criteria given below are satisfied. The activation and nonactivation portions of the mutation assays are usually performed concurrently, but each portion is in fact an independent assay with its own positive and negative controls. The activation or nonactivation assays will be repeated independently, as needed, to satisfy the acceptance and evaluation criteria.

1) The average absolute cloning efficiency of the negative controls (average of the solvent and untreated controls) should be between 70% and 130%. A value greater than 100% is possible because of errors in cell counts (usually ± 10%) and cell division during unavoidable delays between the counting and cloning of many cell cultures. Cloning efficiencies below 70% do not necessarily indicate substandard culture conditions or unhealthy cells. Assay variables can lead to artificially low cloning efficiencies in the range of 50% to 70% and still yield internally consistent and valid results. Assays with cloning efficiencies in this range are conditionally acceptable and dependent on the scientific judgement of the study director. All assays below 50% cloning efficiency are unacceptable.

2) The solvent and untreated negative controls normally have the same growth rates and cloning efficiencies within experimental error. An unusual effect by the solvent therefore indicates an abnormal cell state or an excessive amount of solvent in the growth medium. An assay will be unacceptable if the average percent relative growth of the solvent controls is less than about 70% of the untreated control value.

3) The minimum acceptable value for the suspension growth of the average negative control (average of the solvent and untreated control values) for two days is 8.0. Lower values will render an assay unacceptable for evaluation because of the high frequency of unreliable measurements for both the induced mutant frequency and toxicity of a given treatment. The value of 8 corresponds to three population doublings over the 2-day expression period. The most desirable range for the negative control suspension growth is 12 to 25, since the cells are capable of a 5-fold increase in number under optimal growth conditions for 24 hours.

4) The background mutant frequency (average frequency of the solvent and untreated negative controls) is calculated separately for concurrent activation and nonactivation assays, even though the same population of cells is used for each assay. The activation negative controls contain the S9 activation mix and typically have a somewhat higher mutant frequency than the nonactivation negative controls. For both conditions, the normal range of background frequencies for assays performed with different cell stocks is 5×10^{-6} to 60×10^{-6}. Assays with backgrounds outside this range are not necessarily invalid but will not be used as primary evidence for the evaluation of a test material. These assays can provide supporting evidence.

ASSAY ACCEPTANCE CRITERIA (continued)

5) A positive control is included with each assay to provide confidence in the procedures used to detect mutagenic activity. The normal range of mutant frequencies induced by 0.5 µl/ml EMS (nonactivation assay) is 300 to 800 x 10^{-6}; for 0.3 µl/ml DMN (activation assay) the normal range is 200 to 800 x 10^{-6}. The concurrent background frequencies have been subtracted from these values. These ranges are broad primarily because the effective treatment with these agents is variable between assays. An assay will be acceptable in the absence of a positive control (loss due to contamination or technical error) only if the test material clearly shows mutagenic activity as described in the evaluation criteria. If the test material appears to have no or only weak mutagenic activity, an acceptable assay must have a positive control mutant frequency above the lower limits of the normal range. Assays in which the normal range is exceeded may require further interpretation by the Study Director.

6) For test materials with little or no mutagenic activity, an assay must include applied concentrations that reduce the suspension growth to 10 to 15% of the average solvent control or reach the maximum applied concentrations given in the evaluation criteria. Suspension growth is a combined measure of cell death and reduced growth rates. A 5% relative suspension growth therefore could correspond to 90% killing followed by growth of the survivors at one-half the normal rate for one day and normal growth for the second day. At the other extreme, this condition could be obtained by no killing and complete inhibition of growth for two days. A reasonable limit to testing for the presence of mutagenic action is about 80% to 90% killing of cells. Because of the uncertainty in the actual lethality of treatment in the assay and the fact that mutant frequencies increase as a function of lethality, an acceptable assay for the lack of mutagenic activity must extend to the 10% to 15% relative suspension growth range. There is no maximum toxicity requirement for test materials which clearly show mutagenic activity.

7) An experimental treatment that results in fewer than 2.5 x 10^6 cells by the end of the two-day growth period will not be cloned for mutant analysis.

8) An experimental mutant frequency will be considered acceptable for evaluation only if the relative cloning efficiency is 10% or greater and the total number of viable clones exceeds about 20. These limits avoid problems with the statistical distribution of colonies that can be scored among dishes and allows factors no larger than 10 in the adjustment of the observed number of mutant clones to a unit number of cells (10^6) able to form colonies.

9) Mutant frequencies are normally derived from sets of three dishes for both the mutant colony count and the viable colony count. In order to allow for contamination losses, an acceptable mutant frequency can be calculated from a minimum of two dishes per set if the colony numbers in the two dishes differ by no more than about 3-fold.

ASSAY ACCEPTANCE CRITERIA (continued)

10) The mutant frequencies for five treated cultures are normally determined in each assay. A required number of different concentrations cannot be explicitly stated, although a minimum of three analyzed cultures is considered necessary under the most favorable test conditions to accept a single assay for evaluation of the test material.

MOUSE LYMPHOMA FORWARD MUTATION ASSAY
ASSAY DESIGN NO. 431, EDITION 5

ASSAY EVALUATION CRITERIA

Mutation assays are initiated by exposing cell cultures to a range of concentrations of test material that is expected, on the basis of preliminary toxicity studies, to span the cellular responses of no observed toxicity to growth to complete lethality within 24 hours of treatment. Then five dose levels are usually selected for completion of the mutation assay. The doses are selected to cover a range of toxicities to growth with emphasis on the most toxic doses. An assay may need to be repeated with different concentrations in order to properly evaluate a test material.

The minimum condition considered necessary to demonstrate mutagenesis for any given treatment will be a mutant frequency that is at least 150% of the concurrent background frequency plus 10×10^{-6}. The background frequency is defined as the average mutant frequency of the solvent and untreated negative controls. The minimum increase is based on extensive experience which indicates that assay variability increases with higher backgrounds and the calculated minimum increase as defined above is often a repeatable result; statistical analysis for the confidence limits is not yet available.

The observation of a mutant frequency that meets the minimum criterion for a single treated culture within a range of assayed concentrations is not sufficient evidence to evaluate a test material as a mutagen. The following test results must be obtained to reach this conclusion for either activation or non-activation conditions:

- A dose-related or toxicity-related increase in mutant frequency should be observed. It is desirable to obtain this relation for at least three doses, but this depends on the concentration steps chosen for the assay and the toxicity at which mutagenic activity appears.

- An increase in mutant frequency may be followed by only small or no further increases at higher concentrations or toxicities. However, a decrease in mutant frequency to values below the minimum criterion is not acceptable in a single assay for classifying the test material as a mutagen. If the mutagenic activity at lower concentrations or toxicities was large, a repeat assay will be performed to confirm the mutagenic activity.

- If an increase of about two times the minimum criterion or greater is observed for a single dose near the highest testable toxicity, as defined in the Assay Acceptance Criteria, the test material will be considered mutagenic. Smaller increases at a single dose near the highest testable toxicity will require confirmation by a repeat assay.

ASSAY EVALUATION CRITERIA (continued)

. For some test materials, the correlation between toxicity and applied concentration is poor. The proportion of the applied material that effectively interacts with the cells to cause genetic alterations is not always repeatable or under control. Conversely, measurable changes in frequency of induced mutants may occur with concentration changes that cause only small changes in observable toxicity. Therefore, either parameter, applied concentration or toxicity (percent relative growth), can be used to establish whether the mutagenic activity is related to an increase in effective treatment. A negative correlation with dose is acceptable only if a positive correlation with toxicity exists. An apparent increase in mutagenic activity as a function of decreasing toxicity is not acceptable evidence for mutagenicity.

A test material will be evaluated as nonmutagenic in a single assay only if the minimum increase in mutant frequency is not observed for a range of applied concentrations that extends to toxicity causing 10% to 15% relative suspension growth. If the test material is relatively nontoxic, the maximum applied concentrations will normally be 10 mg/ml (or 10 µl/ml) for water-soluble materials or 1 mg/ml (or 1 µl/ml) for materials in organic solvents. If a repeat assay does not confirm an earlier, minimal response, as discussed above, the test material will be evaluated as nonmutagenic in this assay system.

The ASSAY ACCEPTANCE AND EVALUATION CRITERIA are presented to acquaint the sponsor with the considerations used by the Study Director to determine assay validity and the mutagenic activity of the test material. This presentation may not encompass all test situations, and the Study Director may use other criteria, especially when data from several repeat assays are available, to arrive at a conclusion. The report will provide the reasoning involved when departures from the above descriptions occur.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20989 LBI Assay No. 6837

TYPE of STUDY Mouse Lymphoma assay

This final study report was reviewed by the LBI Quality Assurance Unit on 5-27-83. A report of findings was submitted to the Study Director and to Management on 5-27-83.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Marshall T. Hyman
Auditor, Quality Assurance Unit

JUN - 6 1983

Litton

5516 Nicholson Lane Kensington, Maryland 20895 301 881-5600 • Telex 89-8369

May 31, 1983

Richard C. Davis, Ph.D.
Celanese Corporation
1211 Avenue of the Americas
New York, New York, 10036

RE: FINAL REPORT
Mouse Lymphoma Assay
LBI Assay No. 6837
C-486

Dear Dr. Davis:

Enclosed please find five copies of the above referenced material. This material has been reviewed by our Quality Assurance Unit to ensure compliance with GLP requirements.

If you have any questions or comments concerning the enclosed, please feel free to contact me at (301) 881-5600, extension 122.

Thank you for giving us this opportunity to work with you.

Sincerely,

LITTON BIONETICS, INC.

Brian C. Myhr

Brian C. Myhr, Ph.D.
Director
Department of Molecular Toxicology

BCM/jdc

Enclosures

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Charles E. Moyer, Jr., Ph.D.
Director, Product Safety
Rhône-Poulenc Inc.
CN 7500
Cranberry, New Jersey 08512-7500

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAY 0 8 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and **refer to the reverse side of this page for "EPA Information Requests"** .

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

12059A

Triage of 8(e) Submissions

Date sent to triage: MAY 0 9 1995

NON-CAP CAP

Submission number: 120594

TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: 0 1 2 **pages** 1, 2 **pages** 1, 2, 5, 6

Notes:

Contractor reviewer : _____ **Date:** 4/26/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0992-12059 SEQ. A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Rhone-Poulenc Inc.

SUB. DATE: 09/04/92 OTS DATE: 09/21/92 CSRAD DATE: 03/10/95

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0678) CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: GPTA

CAS#: 52408-84-1

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
(0204)	MUTA (IN VITRO)	01 (02) 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA: NON-CBI INVENTORY
(YES)
CAS SR NO
IN TRIAGE

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES: In Vitro

TOXICOLOGICAL CONCERN:
LOW
(MED)
HIGH

USE:

PRODUCTION:

COMMENTS

6) 8EHQ-92-12059: Rank - medium.

Chemical: glyceryl propoxy triacrylate {α,α',α''-1,2,3-propanetryiltris[ω-[(1-oxo-2-propenyl)oxy]-poly[oxy(methyl-1,2-ethanedyil)]; GPTA; C-486: CAS# 52408-84-1}.

Mutagenicity evaluation of C-486 in the mouse lymphoma forward mutation assay, Litton Bionetics Inc., Kensington MD, dated May, 1983: Positive for gene mutations with a dose response in the L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay in vitro both without and with activation.